Effect of pH-dependent, stationary phase acid resistance on the thermal tolerance of *Escherichia coli* O157:H7†

R. L. Buchanan* and S. G. Edelson

The ability of pH-dependent, stationary phase acid resistance to cross-protect Escherichia coli *O157:H7 against a subsequent lethal thermal stress was evaluated using microbiological media and three liquid foods. Three strains were grown for 18 h at 37°C in acidogenic (TSB+G, final pH 4·6–4·7) and non-acidogenic (TSB-G, final pH 7·0–7·2) media to provide stationary phase cells with and without induction of pH-dependent acid resistance. The cells were then heated in BHI broth (pH 6·0) at 58°C, using a submerged coil apparatus. The TSB+G grown strains had greatly increased heat resistance, with the heating time needed to achieve a five-log inactivation, being increased two- to four-fold. The z-values of TSB+G and TSB-G grown cells were 4·7°C and 4·3°C, respectively. Increases in heat resistance with TSB+G-grown* E. coli *O157:H7 were also observed using milk and chicken broth, but not with apple juice. However, cross-protection was restored if the pH of the apple juice was increased from 3·5 to 4·5. The data indicate that pH-dependent acid resistance provides* E. coli *O157:H7 with cross-protection against heat treatments, and that this factor must be considered to estimate this pathogen's thermal tolerance accurately.*

Introduction

Cooking remains the primary means by which pathogenic micro-organisms are eliminated from foods. The degree of microbiological control achieved by a cooking step is dependent on numerous factors. In addition to the time and temperature of cooking, effectiveness is dependent on the inherent thermal resistance of the micro-organism and the compositional and physical characteristics of the food. There are often competing organoleptic considerations. Excessive cooking can lead to the development of off-flavors, undesirable changes in texture and other losses of desirable sensorial or nutritional food characteristics. In such instances, selection of cooking times and temperatures involves process optimization, i.e., maximization of microbial destruction and minimization of organoleptic deterioration. A key to optimization of a cooking step is accurate information on the target pathogen's thermal resistance. Under-estimating its thermal

*Corresponding author. Current address: US, Food and Drug Administration, Center of Food Safety and Applied Nutrition, 200 C-Street, SW, Washington, DC 20204, USA. Fax: 202 205-4970.
E-mail: rbuchana@bangate.fda gov.

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resistance increases the risk that a sufficient portion of an initial pathogen population may persist after cooking. Conversely, overestimating resistance leads to heat treatments that may cause unnecessary quality losses.

The thermal resistance of various foodborne pathogens, such as *Salmonella typhimurium, Listeria monocytogenes* and *Escherichia coli,* can be influenced by the micro-organisms' responses to other physiological stresses. Acid tolerance and acid resistance/habituation responses can produce cross-protection against several other stresses including heating (Bearson et al. 1997). For example, acid-adapted *S. typhimurium* had increased resistance to heating, osmotic stress, lactoperoxidase and crystal violet (Leyer and Johnson 1993; Lee et al. 1994). Cross-protection effects due to acid adaptation have also been noted with *L. monocytogenes* (Farber and Pagotto 1992, Lou and Yousef 1996) and *E. coli* (Goodson and Rowbury 1991, Buchanan et al. 1999). Several different types of acid adaptation have been identified in various micro-organisms, and vary with the growth stage of the organism. Stationary phase cells are typically more resistant than exponential phase cells. In *E. coli* O157:H7, the increased acid resistance and thermal resistance of stationary phase cells has been attributed to the expression of *rpoS*-regulated genes (Cheville et al. 1996). In addition to this pH-independent resistance, pH-dependent acid resistance has also been observed (Small et al. 1994, Buchanan and Edelson 1996), and provides cross protection against ionizing irradiation (Buchanan et al. 1998, 1999). The objective of the current study was to determine in microbiological media and liquid foods whether the induction of pH-dependent acid resistance in *E. coli* O157:H7 also increases the pathogen's thermal resistance.

Materials and Methods

Micro-organisms

Escherichia coli O157:H7 strains Ent-C9490, A9124-C1, and SEA13B88 were used in the study. The sources and maintenance of these strains have been described previously (Buchanan and Edelson 1996, 1998).

Inoculum

The strains were grown individually in 125-ml Erlenmeyer flasks containing 25 ml of tryptic soy broth (Difco, Detroit, Michigan, USA) with 1% dextrose (TSB+G) or tryptic soy broth without dextrose (TSB-G; Difco). The flasks were inoculated with 0·1 ml of a stock culture and incubated for 18 h at 37°C without agitation. TSB+G is acidogenic, producing cultures that have a final pH of approximately 4·6–4·7 and that are induced for pH-dependent, stationary phase acid resistance (Buchanan and Edelson 1996). Conversely, TSB-G is non-acidogenic, yielding cultures that have a final pH of 7·0–7·2 and that are not induced for pH dependent acid resistance.

Preparation of materials for thermal resistance determinations

Brain–heart infusion (BHI; Difco) was prepared and acidified to pH 6·0 using HCl. The BHI was dispensed in 9·5-ml portions to test tubes (16 × 125 mm), sealed with plastic caps and sterilized by autoclaving for 15 min at 120°C. The pH of representative tubes was determined after autoclaving to verify that the pH was within 0·1 units of the target.

UHT non-fat milk (pH 6·5), canned fat-free chicken broth (pH 6·1) and bottled clarified apple juice (pH 3·5) were purchased at a local retail market. These liquid foods were transferred in 9·5-ml portions to sterile test tubes. pH-modified apple juice was handled in the same manner. Its pH was modified by adding solid sodium hydroxide to achieve pH values of 4·5, 5·5 and 6·5.

Determination of thermal resistance

The thermal resistance of TSB+G and TSB−G grown cells of the three strains was determined in BHI using a submerged coil apparatus (Cole and Jones 1990) preequilibrated to 58°C. The submerged coil apparatus can achieve highly accurate and reproducible thermal inactivation determinations. However, it is limited to microbiological media or liquid foods. A 9·5-ml BHI tube was inoculated with 1·1 ml of either a TSB+G or TSB–G 18-h culture.

Immediately after mixing, 0·6 ml was transferred to a prechilled 5·4-ml dilution blank of sterile 1% buffered peptone water to produce a 10^{-1} dilution. After mixing the blank was placed on ice. The remainder of the inoculated BHI was injected into the submerged coil apparatus. At designated times, the apparatus dispensed 0·6-ml samples into sterile vials. The dispensed samples were immediately transferred to 5·4-ml dilution blanks, and placed on ice until survivors could be enumerated. This was typically within 1–2 h. Preliminary trials indicated that holding the samples on ice for this period did not alter observed thermal resistance values. At least three separate heating trials were performed for each growth medium for each strain.

The 10^{-1} dilutions of heated samples were diluted further as needed using 9·9 ml dilutions blanks and then surface plated on duplicate BHI agar (Difco) plates using a spiral plater (Spiral Biotech, Bethesda, Maryland, USA. All plates were incubated for 18–24 h at 37°C and then enumerated using an automatic plate counter (Spiral Biotech).

The heat resistance of *E. coli* O157:H7 SEA13B88 in liquid foods was determined as described above for BHI. This strain was selected because it was the most acid-resistant of the three strains and had the greatest thermal resistance after induction of pH-dependent acid resistance.

Analyses

Inactivation curves were generated by fitting the data to the two-phase linear model of Buchanan et al. (1994):

$$t \leq t_L : N = N_0 \qquad (1)$$

$$t > t_L : N = N_0 + s(t - t_L) \qquad (2)$$

where t = duration of heating (s), t_L = duration of lag period before inactivation is initiated (s), N_0 = initial number of bacteria (log (cfu ml^{-1})), N = number of surviving bacteria at time t (log(cfu ml^{-1})), s = slope of the survivor curve (log(cfu ml^{-1}))s^{-1}. The data were fitted using the curve fitting software ABACUS (Damert 1994). D-values for the inactivation portion of the curves were calculated by taking the negative reciprocal of s. Time to a $5D$ inactivation values (t_{5D}) were calculated using the equation:

$$t_{5D} = t_L + 5D \qquad (3)$$

The negative reciprocals of the slopes of the linear regressions of the $\log_{10}$ of D-values vs temperature were used to calculate z-values.

Results

Inactivation in BHI

Growth of the three strains of *E. coli* O157:H7 in TSB+G and TSB−G for 18 h at 37°C consistently provided stationary phase cells with final pH values of 4·6–4·7 and 7·0–7·2, respectively. Representative survivor curves for *E. coli* O157:H7 strain SEA13B88 grown in acidogenic TSB+G and non-acidogenic TSB−G and then heated in BHI at 58°C using a submerged coil apparatus are depicted in Fig. 1. The TSB-G grown cells displayed linear inactivation kinetics. The response of strains Ent-C9490 and A9124-C1 were similar. The inactivation kinetics of TSB+G grown cells were non-linear, having an extended lag period (t_L) prior to the initiation of inactivation. The magnitude of this shoulder was approximately twice that of the strains' D-values (Table 1). The D-values for TSB+G grown cells of strains SEA13B88 and

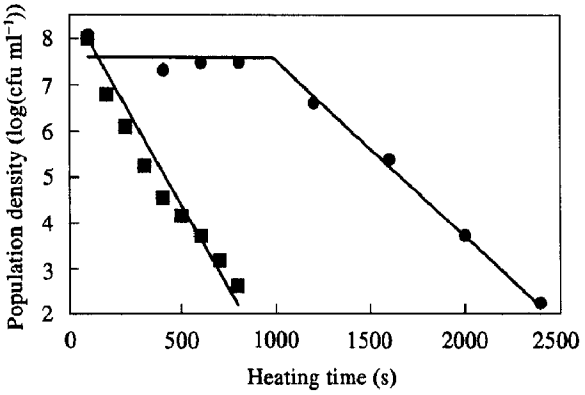

Figure 1. Representative example of the survivor curves observed with *E. coli* O157:H7 (SEA13B88) grown in acidogenic (●) and non-acidogenic (■) medium and then heated in brain–heart infusion broth (pH 6·0) at 58°C.

Table 1. Effect of prior growth in acidogenic (TSB+G) and non-acidogenic (TSB−G) media on the thermal resistance of three strains of *Escherichia coli* O157:H7

Strain	Growth medium	n	t_L-value (s)[a]	D-value (s)	t_{5D}-value (s)
Ent-C9490	TSB+G	9	719·6	324·6	2342·5
			(66·5)	(34·1)	(159·3)
	TSB−G	6	14·3	123·7	632·8
			(17·2)	(10·2)	(50·6)
SEA13B88	TSB+G	6	755·1	334·3	2426·6
			(131·6)	(42·6)	(193·3)
	TSB−G	6	0·0	149·1	745·3
				(13·7)	(68·3)
A9124-C1	TSB+G	12	595·9	262·6	1909·0
			(230·6)	(51·6)	(269·2)
	TSB−G	12	0·0	223·4	1116·9
				(77·8)	(388·8)

[a]Mean (standard deviation).

Ent-C9490-C1 were approximately double that of the TSB−G cells. The D-values for TSB+G and TSB−G grown cells of strain A9124-C1 were similar. Using t_{5D}-values as a measure of the overall ability of *E. coli* O157:H7 to survive heating, pH-dependent, stationary phase acid resistance increased thermal resistance by two- to four-fold.

Strain SEA13B88 was used to determine the effects of prior growth conditions on the thermal resistance of *E. coli* O157:H7 over the temperature range 56–62°C. Acid resistance cross-protected the micro-organism at each of the heating temperatures tested (Fig. 2). The TSB+G grown cells consistently had a lag period before inactivation, the magnitude of which was inversely related to heating temperature (Fig. 2(a)). Again, this lag period was not evident with TSB−G grown cells. The $\log_{10}(D$-values) (Fig. 2(b)) and $\log_{10}(t_{5D}$-values) (Fig. 2(c)) for TSB+G and TSB−G grown cells were linearly related to heating temperature, with the values for the TSB+G cells being proportionally greater than the TSB−G cells over the temperature range. Induction of pH-dependent, stationary phase acid resistance had little effect on the pathogen's z-value, compared to pH-independent stationary phase acid resistance alone. The z-values for TSB+G and TSB−G grown cells were 4·73° and 4·25°C, with standard deviations of 0·11 and 0·05°C, respectively.

Inactivation in liquid foods

Chicken broth, UHT milk, and apple juice were used to determine if the cross-protection provided by pH-dependent, stationary phase acid resistance also occurred in foods. The evaluation was limited to liquid foods heated at 58°C so that the submerged coil apparatus could be employed, thus allowing the results to be compared directly with BHI derived data. The response in UHT milk (Fig. 5) was similar to that observed with BHI. Prior culturing in acidogenic TSB+G increased the heat resistance of *E. coli* O157:H7 substantially. Again, the increased thermal resistance was the result of both increases in D-values and the presence of a extended lag period. As with BHI, inactivation kinetics for TSB−G grown cells were linear. The t_L-values, D-values and t_{5D}-values for *E. coli* in milk were 0 s, 132 s and 668 s, for TSB−G-grown cells, and 641 s, 359 s and 2435 s for TSB+G-grown cells. The response in chicken broth (Fig. 3) also indicated increased thermal resistance for TSB−G grown cells, but an increased t_L was not clearly discernable. This lack of a lag period for the TSB+G grown cells may be an artifact of the sampling times selected. The D-values and t_{5D}-values for chicken broth were 146 s and 731 s for TSB-G-grown cells, and 404 s and 2022 s for TSB+G grown cells.

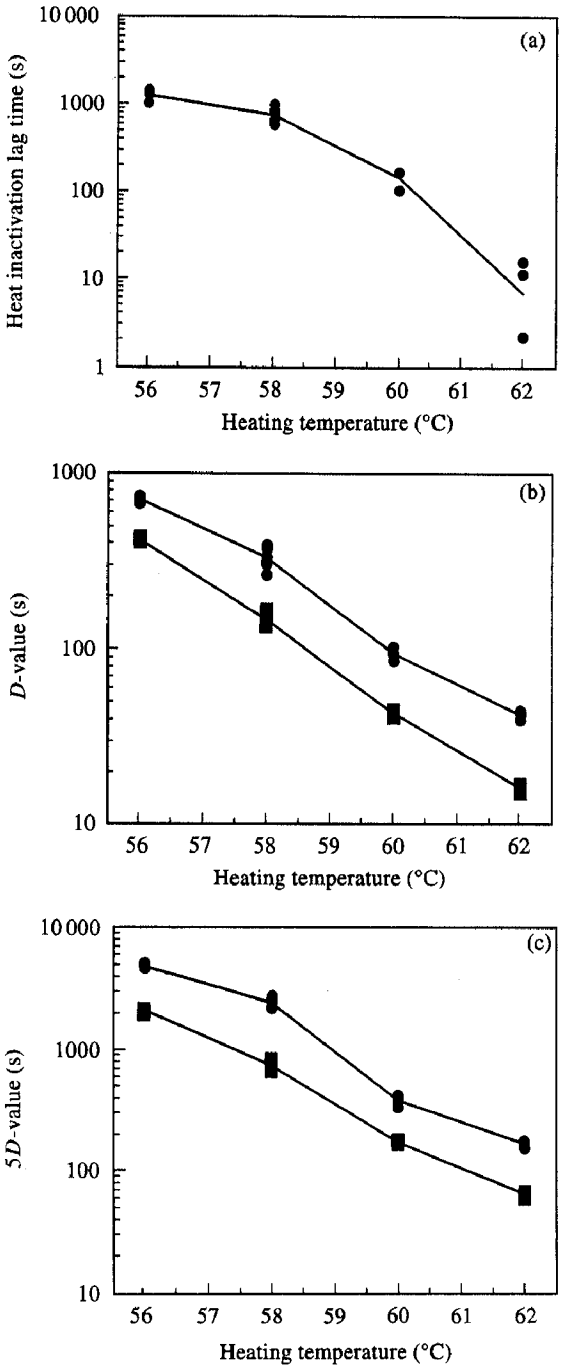

Figure 2. t_L-values (a), D-values (b), and t_{5D}-values (c) for *E. coli* O157:H7 (SEA13B88) grown in acidogenic (●) and non-acidogenic (■) medium and then heated in brain–heart infusion broth (pH 6·0) at 56, 58, 60 and 62°C. Each point is the average of at least three independent heating trials.

Induction of pH-dependent, stationary phase acid resistance did not produce equivalent cross protection in apple juice (Fig. 4). In this acidic food, the D-value for TSB+G grown cells was slightly less than that for the TSB-G cells, and their t_{5D}-values were equivalent. While enhanced heat resistance did not occur with the TSB+G grown cells, their survivor curves did appear to have a small, though statistically discernable deviation from the linear inactivation kinetics associated with the TSB−G grown cells.

Since a major difference between apple juice and the other liquid foods is its pH, the thermal resistance of *E. coli* O157:H7 in unmodified apple juice was compared against apple juice adjusted to pH 4·5, 5·5 and 6·5 (Table 2). The enhanced thermal resistance associated with TSB+G-grown *E. coli* was evident at each of the higher pH values. There was no corresponding increase in the heat resistance of TSB−G grown cells at the higher pH values. The increased resistance in the pH modified apple juice was due largely to increases in D-values instead of the duration of the lag periods.

Discussion

Optimized thermal processes assure that foods have received heat treatments sufficient to reduce, by a desired degree, the probability that a target micro-organism survived while simultaneously minimizing deleterious organoleptic effects. This requires accurate data on the extent, characteristics, and biovariability of the micro-organism's thermal resistance. It has been long recognized that environmental factors such as water activity and pH can affect microbial thermal resistance, and stationary phase cells are more resistant to a number of stresses including heat (Jay 1996). In the latter case, resistance has been associated with the expression of new genes, regulated by the alternative sigma factor that is encoded by the *rpoS* locus (Cheville et al. 1996). In addition, there is increasing information available concerning other adaptive capabilities possessed by different foodborne pathogens that influence their thermal resistance. For example, heat shock or growth at elevated temperatures increases the thermal resistance of *Salmonella* (Mackey and Derrick, 1987, 1990; Bunning et al. 1990; Humphrey et al. 1993, Xavier and Ingham 1997),

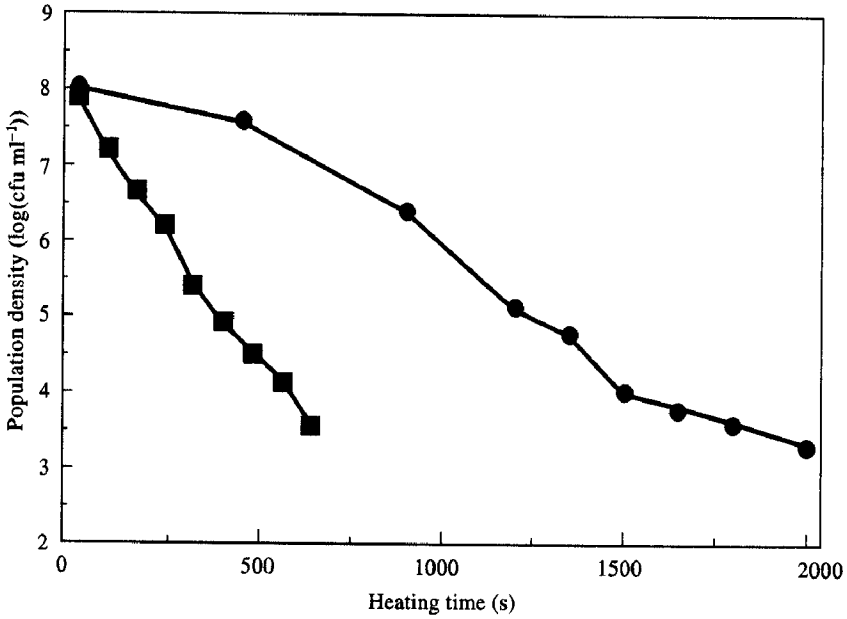

Figure 3. Survivor curves for *E. coli* O157:H7 (SEA13B88) grown in acidogenic (●) and non-acidogenic (■) medium and then heated in canned fat-free chicken broth (pH 6·1) at 58°C. Each point is the average of at least three independent heating trials.

L. monocytogenes (Bunning et al. 1990, Knabel et al. 1990, Pagán et al. 1997), and *E. coli* (Murano and Pierson 1993, Gadzella and Ingham 1994, Jackson et al. 1996, Juneja et al. 1998). Microbial adaptation responses to one stress can lead to cross protection against another stress. Increased thermal tolerance resulting from bacterial responses to exposure to an acidic environment has been demonstrated in *L. monocytogenes* (Farber and Pagotto 1992, Lou and Yousef 1996) and *S. typhimurium* (Leyer and Johnson 1993); however, the current study

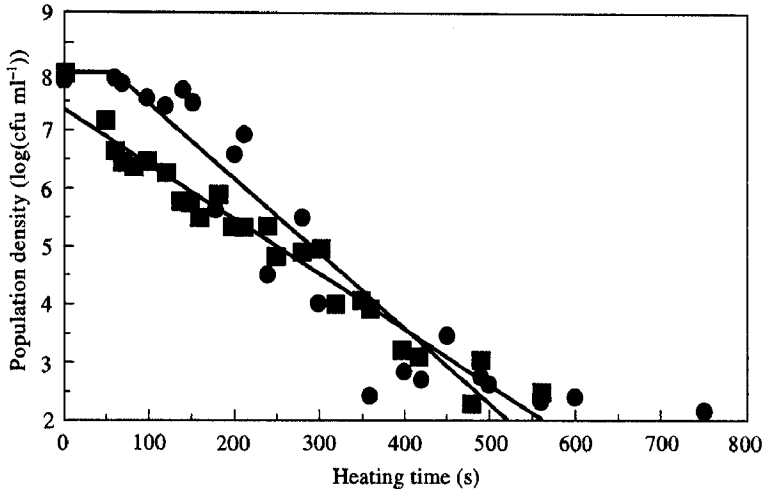

Figure 4. Survivor curves for *E. coli* O157:H7 (SEA13B88) grown in acidogenic (●) and non-acidogenic (■) medium and then heated in bottled, clarified apple juice (pH 3·5) at 58°C. The data are the consolidation of twelve independent heating trials, with each point representing the average of at least three replicates.

Table 2. Effect of pH modification on the thermal resistance of *Escherichia coli* O157:H7 (SEA13B88) heated in apple juice at 58°C

Growth medium[a]		pH of apple juice			
		3·5	4·5	5·5	6·5
TSB+G	t_L[b]	107·4	304·5	171·2	163·9
	D	74·1	196·1	277·8	238·1
	t_{5D}	477·7	1284·9	1560·1	1354·4
TSB−G	t_L	0·0	0·0	0·0	0·0
	D	125·0	112·4	137·0	120·5
	t_{5D}	625·0	561·8	685·0	602·4

[a]Cells grown in acidogenic tryptic soy broth + 1% dextrose (TSB+G) (final pH: 4·6–4·7) or non-acidogenic tryptic soy broth without dextrose (TSB − G) (final pH: 7·0–7·2) for 18 h at 37°C prior to transfer to apple juice.
[b]Abbreviations: t_L: lag period (s) prior to initiation of thermal inactivation; D:D-value (s); t_{5D}: time to a 5-$\log_{10}$ decline in population density (s); $5\text{-}D=t_L+5*D$.

appears to be the first detailed examination of the phenomenon in enterohemorrhagic *E. coli*.

The general effect of inducing pH-dependent stationary phase acid resistance on the microorganism's thermal tolerance was similar among the strains; there was a shift from linear to nonlinear inactivation kinetics (Fig. 1, Table 1). There are several potential explanations for the presence of a shoulder in a survivor curve. One strong possibility is that the cell has synthesized a constituent that spares the cellular component that becomes inactivated during heating. Until the cellular pool of the sparing agent is eliminated,

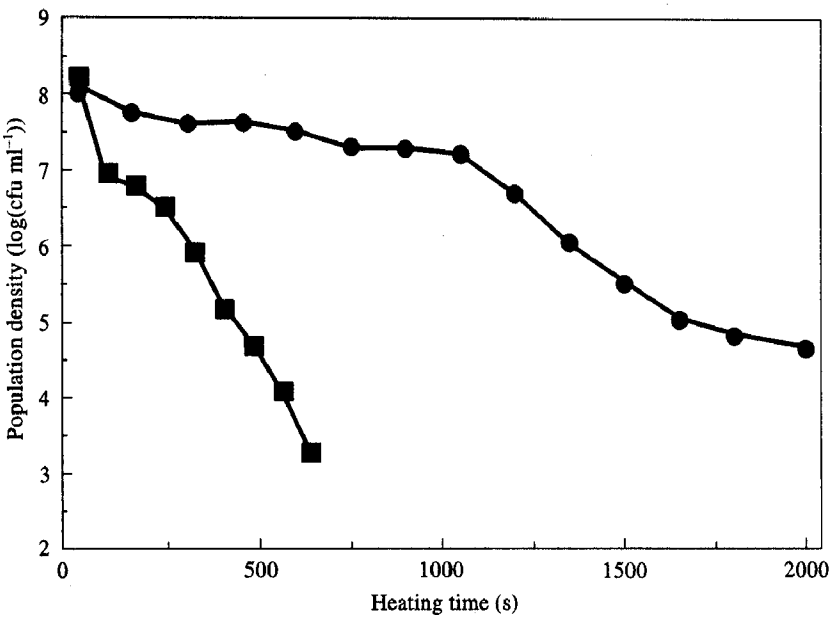

Figure 5. Survivor curves for *E. coli* O157:H7 (SEA13B88) grown in acidogenic (●) and non-acidogenic (■) medium and then heated in UHT non-fat milk (pH 6·5) at 58°C. Each point is the average of at least three independent heating trials.

Table 3. Comparison of *D*-values[a] for *Escherichia coli* O157:H7 observed in the current study with those reported in the literature

Reference/Menstruum	D_{58} (s)	*z*-value (°C)	t_{5D} (s)	Comments
Ahmed et al. (1995)				Grown in BHI for 24 h
Ground chicken				
low fat	113	4·5	567	
high fat	122	4·4	609	
Ground turkey				
low fat	121	4·7	441	
high fat	88	4·4	606	
Ground beef				
low fat	162	4·8	810	
medium fat	191	4·4	954	
high fat	241	4·4	1203	
Pork sausage				
low fat	88	4·7	441	
medium fat	108	4·7	540	
high fat	151	4·6	753	
Splittstoesser et al. (1996)				Grown in TSB for 24 h. Values for pH 4·0 and 4·5 estimated from D_{52} and *z*-values.
Apple juice				
pH 3·6	60	4·8	300	
pH 4·0	77	4·8	384	
pH 4·5	150	4·8	750	
Line et al. (1991)				
Ground beef				Grown in TSB-YE broth for 48 h
low fat	161	4·6	804	
high fat	298	4·3	1488	
Doyle and Schoeni (1984)				Grown in TSB to late stationary phase.
Ground beef	204	4·1	1020	
Juneja et al. (1997)				Grown in BHI for 24 h
Ground beef	244–245	4·9–6·0	1173–1227	
Ground chicken	187–192	5·8–6·8	933–960	
Orta-Rumirez et al. (1997)				Grown in TSB for 24 h
Ground beef	386	5·6	1932	
Thippareddi et al. (1995)				Induced to acid resistance Grown in TSB and TSB+G
Peptone water				
strain # 1	388	4·8[b]	1941	
strain #2	232	4·8[b]	1161	
Jackson et al. (1996)				Grown to stationary phase in TSB at three different growth temperatures (GT), and then heated. Evidence of nonlinear kinetics with some cultures. t_{5D} values estimated using D_L only.
TSB				
GT: 23°C	98	4·8[b]	492	
GT: 30°C	222	4·8[b]	1110	
GT: 37°C	303	4·8[b]	1515	
Murano and Pierson (1993)				Grown in TSB for 6 h
condition #1	114	4·8[b]	570	non-shocked/aerobic recovery
condition # 2	239	4·8[b]	1194	heat-shocked/aerobic recovery
condition # 3	260	4·8[b]	1302	non-shocked/anaerobic recovery
condition # 4	316	4·8[b]	1578	heat-shocked/anaerobic recovery
Current Study: TSB-G Grown:				Grown in TSB-G for 18 h
BHI (pH 6·0)				
strain #1	124		633	
strain #2	149	4·3	745	
strain #3	223		1117	
UHT Milk	132		668	
Chicken broth	146		731	

Table 3. (Continued)

Reference/Menstruum	D_{58} (s)	z-value (°C)	t_{5D} (s)	Comments
Apple juice				
pH 3·5	125		625	
pH 4·5	112		562	
pH 5·5	137		685	
pH 6·5	121		602	
Current Study: TSB+G Grown:				Grown in TSB+G for 18 h
BHI (pH 6·0)	325		2343	
strain #1	334	4·7	2427	
strain #2	2636		1909	
strain #3	359		2435	
UHT milk	404		2022	
Chicken broth				
Apple juice	74		478	
pH 3·5	196		1285	
pH 4·5	278		1560	
pH 5·5	238		1354	
pH 6·5				

[a]Time in seconds needed to achieve a 90% reduction in population density when heated at 58°C.
[b]Cultures manipulated to increase their thermal tolerance.

inactivation is not initiated. Shifts to nonlinear inactivation kinetics have also been noted when *L. monocytogenes* is heat-shocked prior to exposure to a lethal heat treatment (Pagán et al. 1997). These investigators observed that survivor curve shoulders were eliminated when heat-shocked cells were plated on media that did not support the repair of injured cells, and hypothesized that protective effect was the result of the synthesis of heat shock proteins. Exposure to acidic environments does induce the synthesis of sets of characteristic stress proteins in different foodborne pathogens, and this has been hypothesized to contribute to cross-protection effects associated with acid resistance (Foster 1995). Induction of acid resistance in *E. coli* O157:H7 has been also reported to increase levels of cell membrane stabilizing cyclopropane fatty acid containing phospholipids (Brown et al. 1997). Induction of pH-dependent stationary phase acid resistance has been reported to increase the ability of *E. coli* to survive acid injury (Buchanan and Edelson 1996, 1998). This effect was most evident with highly acid resistant enterohemorrhagic strains, and was hypothesized to involve protection of the cell membrane. It is worth noting that heat shock response in *S. enteritidis* increases its acid resistance (Humphrey et al. 1993).

While all three strains tested display a shift to nonlinear kinetics, differences were observed among the isolates (Table 1). In addition to having substantial t_L values, TSB+G grown Ent-C9490 and SEA13B88 had greater than twofold increases in D-values. This is in contrast to A9124-C1, which had little if any increase in its D-value. The reason for this difference will require further study, but may be related to the inherent acid resistance characteristics of the isolates. When grown in TSB−G, strain A9124-C1 is substantially less acid resistant than the others, but showed a substantially greater increase in relative acid resistance when grown in TSB+G (Buchanan and Edelson 1996, 1998). Thippareddi et al. (1995) reported that prior growth in TSB +1% dextrose did not increase the thermal resistance of two strains of *E. coli* O157:H7. However, their comparison was based on standard TSB which contains 0·25% dextrose. TSB is sufficiently acidogenic that their control cells may also have been induced to pH-dependent stationary phase acid resistance. The D-values

reported by Thippareddi et al. (1995) are consistent with what would be expected with cross-protected cells.

While induction of pH-dependent stationary phase acid resistant influenced the absolute thermal resistance of strain SEA13B88, it did not appear to have a great impact on how heating temperature affected the micro-organism's relative heat resistance (Fig. 2). The *z*-values of TSB+G- and TSB−G-grown cells were similar, despite the former prior growth conditions producing substantially more heat resistant cells. This suggests that the underlying mechanism for thermal inactivation is the same for TSB+G- and TSB−G-grown cells once first order inactivation kinetics commenced, at least in relation to the effect of heating temperature. Like the linear relationship between heating temperature and log (*D*-values), the log (t_{5D}) was linearly related to heating temperature despite that shift to non-linear inactivation kinetics when grown in an acidogenic medium. This reflects the inverse relationship between t_L values and heating temperature. This correlation between the duration of the shoulder and processing temperature has been previously described for *L. monocytogenes* (Bhaduri et al. 1991).

The increased resistance of TSB+G grown *E. coli* O157:H7 SEA13B88 when heated in canned chicken broth (Fig. 3) and UHT milk (Fig. 5) demonstrates that, at a minimum, the phenomenon can occur in liquid food products. While it will require experimental verification, there is no reason to anticipate that a similar response would not occur in solids foods that had a pH $\geq$ 4·5. The lack of increased heat resistance in apple juice is of particular interest (Fig. 4). While the t_{5D} values for TSB+G- and TSB−G-grown cells were similar, the TSB+G-grown cells still displayed a shift to nonlinear inactivation kinetics. This, in combination with the restoration of cross-protection when the pH of the apple juice was neutralized (Table 2), suggest that growth in acidogenic TSB+G was inducing cross-protection but it was not realized due to a characteristic of the mechanism for induced heat resistance. It has long been observed that the heat resistance of foodborne pathogens decrease at pH values < 4·0–4·5. It would be of interest to determine if this is due to a change in basal heat resistance or the elimination of acid resistance-associated cross-protection. The observation that the *D*-values for TSB−G-grown cells were similar when heated in apple juice over a pH range of 3·5–6·5 (Table 2) suggest that loss of cross-protection may play a role. That the behavior of *E. coli* in apple juice was associated with the mechanism of heat resistance and not a lack of induction of cross-protection is supported further by the observation that prior growth in TSB+G increased the radiation resistance of *E. coli* O157:H7 SEA13B88 in the same apple juice (Buchanan et al. 1998b). Although both are induced by pH-dependent stationary phase acid resistance, the mechanisms for cross-protection-enhanced thermal tolerance and irradiation resistance are obviously different. Humphrey et al. (1993) concluded that while heat shock enhanced the thermal tolerance and acid resistance of *S. enteritidis*, the mechanisms underlying the responses were different.

The current study demonstrates that the pH-dependent stationary phase can substantially increase the thermal tolerance of enterohemorrhagic *E. coli*. If not considered when developing thermal process specifications this could lead to insufficient heating times. As an extreme example, consider a thermal process designed to achieve a five-log inactivation of *E. coli* O157:H7 based on the D_{58}-value observed for TSB-G grown cells in UHT milk (Fig. 5). If contaminating *E. coli* actually had the heat resistance observed with TSB+G, the cells would still be in the t_L period when the process was completed. As a means of assessing if current estimates of the thermal tolerance of *E. coli* O157:H7 would be sufficient to overcome acid resistance-induced thermal cross-protection, the current results were compared with published *D*-values for stationary phase cells (Table 3). In addition, t_{5d} values were calculated to more accurately estimate the effect of nonlinear inactivation kinetics. These results are also depicted graphically to allow easier comparison of relative heat resistances (Fig. 6). In reviewing reported values, it is apparent that there is a great deal of variation among the *D*-values reported for different commodities. Likewise, there was substantial differences in the conditions under which the cells were

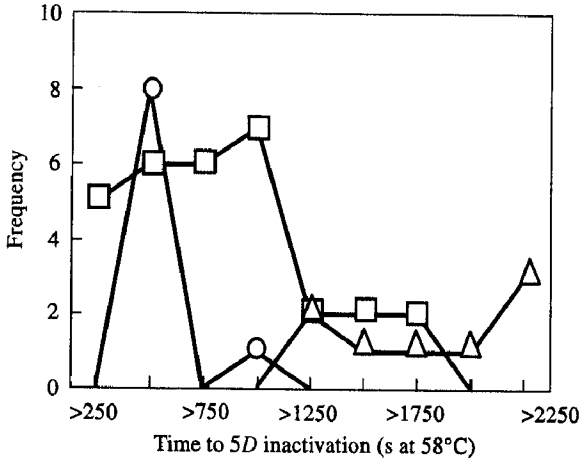

Figure 6. Comparison of t_{5D} values from current study and those reported in the literature. Literature values (□), current study, TSB-G-grown cells (○), current study, TSB+G-grown cells (△).

grown. The TSB-G grown cells had *D*-values and t_{5D} values that were comparable with those reported in the literature. Conversely, the time to achieve a 5-D inactivation of TSB+G grown cells was greater than reported values. Further, the literature values that showed extended t_{5D} values were those that either the investigators had enhanced heat resistance or had grown *E. coli* in TSB. Based on the results of Thippareddi et al. (1995), TSB may have enough dextrose (0·25%) to elicit at least some cross-protection. It is worth noting that to the best of our knowledge the thermal processing times and temperatures for inactivation of enterohemorrhagic *E. coli* currently recommended do not take into account increases in thermal resistance due to cross-protection.

It is apparent that unless pH-dependent cross-protection is taken into account, it is likely that the thermal tolerance of acid resistant *E. coli* would be underestimated. This is amplified by the fact that one must assume that *E. coli* O157:H7 present in food are acid-resistant, since the pathogen's normal niche appears to be the rumen and large intestine of herbivores. The current study suggests that current heating recommendations for the elimination of enterohemorrhagic *E. coli* from different foods may have to be reexamined to ensure that they are based on the pathogen being in its most thermally resistant state. Finally, our increasing understanding of the wide range of factors that can affect the thermal resistance underscores the need to standardize how thermal resistance determinations should be conducted.

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